EFFECTS OF OUTBREEDING DEPRESSION ON MERISTICS AND BILATERAL ASYMMETRY IN HYBRIDS OF SPATIALLY SEPARATED POPULATIONS OF PINK SALMON (*ONCORHYNCHUS GORBUSCHA*)

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ABSTRACT

Different populations of a species distributed over diverse conditions adapt to their local environments to improve their ability to survive or reproduce. Intraspecific hybridization can alter the locally adapted population, resulting in reduced fitness, causing outbreeding depression. Manifestations of outbreeding depression in Pacific salmon include decreases in survival, fitness, and/or fitness-related traits. Many animals have paired morphological structures, resulting from canalization during development, which promote the animal's fitness; more symmetrical individuals often have faster growth, higher fecundity, or better survival. Meristic traits, such as the number of gill rakers in fish, can be easily determined. This study examined the potential effects of outbreeding depression on morphological meristic characteristics. Variation in fish size and meristic counts of returning F_1 and F_2 hybrids of spatially separated populations of pink salmon was compared to those of controls. There was no evidence for increased fluctuating asymmetry in hybrids. Directional asymmetry was significant for branchiostegals and pectoral fin ray counts. No single character consistently had sire or interaction effects except gill rakers; the few significant effects probably result from maternal environment effects. Canalization of bilateral asymmetry seems to be relatively unaffected by outbreeding depression.

SOUTHWORTH

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ACKNOWLEDGMENTS

For this thesis I participated in the collection of the final year's meristic counts and conducted all of the data analysis, with the assistance of A.J. Gharrett. I also directed recovery efforts for one generation of fish and wrote the manuscript. A.J. Gharrett and W.W. Smoker designed and set up the experiments. I.A. Wang maintained the first three generations of fish, oversaw clipping in these years, and directed recoveries of adult fish during the first returns. S.E. Gilk maintained one generation of pink salmon and directed recovery efforts for one generation of fish. S.G. Taylor is manager of the Auke Creek weir and hatchery, and was responsible for capturing the returning fish at the weir.

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Effects of outbreeding depression on meristics and bilateral asymmetry in hybrids of spatially separated populations of pink salmon (*Oncorhynchus gorbuscha*) [1]

INTRODUCTION

Meristic traits, countable characters such as the number of gill rakers, fin rays, or vertebrae, are fundamental characters used in taxonomic studies, and are often considered the most reliable of taxonomic characteristics of fishes because they can be easily determined (Tåning 1952; Barlow 1961). Factors that affect larval growth (e.g., temperature, dissolved oxygen, salinity, or food availability) can affect meristic characters (Tåning 1952; Moyle and Cech Jr. 2000). The number of a meristic character is usually close to a phenotypic norm; and paired structures are usually similar, even when they are influenced by environmental variation. The process that buffers complex development is referred to as canalization (Waddington 1942). Changes in the phenotypic norm of a meristic character require major alterations of the developmental pathway either as a result of changes in selection pressure, so the norm is no longer associated with maximum fitness, or as a consequence of the introduction of new genetic variability capable of altering the developmental process despite canalization (Waddington 1942).

Many animals (for example, fish, birds, and humans) have paired morphological structures which promote the animal's fitness (Møller 1997). Although development involves the coordination of many genes, the structures should be mirror images

[1] C. L. Hoover, S. E. Gilk, I. A. Wang, W. W. Smoker, M. D. Adkison, and A. J. Gharrett. A manuscript prepared for submission to Transactions of the American Fisheries Society.

(bilaterally symmetrical) because stability presumably reflects coadaptation of the entire genome in producing the phenotype (Leary and Allendorf 1989; Graham 1992). However, biological systems do not consistently achieve perfect bilateral symmetry, even under ideal environmental conditions, because minor inconsistencies during development can cause small deviations in developmental pathways (Palmer and Strobeck 1992). Such deviations are relatively common, and can be characterized by the frequency distribution of right (R) minus left (L) measurements (Van Valen 1962). Conceptually, three types of bilateral asymmetry are fluctuating asymmetry, directional asymmetry, and antisymmetry. Directional asymmetry is characterized by a mean significantly different from zero (e.g., position of the heart in mammals), whereas antisymmetry arises as a genetic predisposition of an individual towards asymmetry but with no specific bias to left or right (e.g., the claws of lobsters and crabs) (Palmer and Strobeck 1986; Berg et al. 1997).

Fluctuating asymmetry (FA), which refers to random, independent differences in trait number, size, shape, or other feature between the right and left sides, is characterized by a symmetrical distribution of the individual right minus left (R - L) values around a mean of zero, and has been used as an indicator of developmental stability or homeostasis (Van Valen 1962; Soulé 1979; Palmer and Strobeck 1992). Symmetrical individuals may have faster growth, higher fecundity, or better survival than more asymmetrical individuals (Møller 1997). Variation of FA has been examined for a variety of traits and in a number of organisms (reviewed in Palmer and Strobeck 1986). Usually FA occurs at low levels, and elevation in FA may reflect disruption in development, either by internal

genetic factors, such as inbreeding or outbreeding, or by external factors such as environmental disturbance or pollution (Wilkins et al. 1995; Bryden and Heath 2000). Consequently, increases in levels of asymmetry may indicate that a population is under stress as a result of loss of genetic variation or an inhospitable habitat. The perturbations in development, reflected by FA, may be a useful indicator of organisms subjected to stress (Leary and Allendorf 1989).

In addition to environmental stress, genetic stressors contribute to increased FA. One hypothesis is that low heterozygosity may result in increased FA (Clarke 1993). The explanation is that heterozygosity buffers the organism from disturbances affecting normal developmental processes and pathways. Genetic stress may also result from the disruption of coadapted gene complexes that are intrinsic to developmental stability (Clarke 1993; Hochwender and Fritz 1999). Hybridization both increases heterozygosity and disrupts coadapted gene complexes (Graham 1992), and its effects on FA have been widely examined to determine which hypothesis more accurately explains the basis for developmental stability (reviewed in Hochwender and Fritz 1999). Wilkins et al. (1995) proposed that the sequential nature of developmental events suggests that epistatic interactions may be more important than heterozygosity at single loci.

Outcomes of hybridization are heterosis, outbreeding depression, or no effect; and the effects may change in sequential generations. Heterosis, or hybrid vigor, results from the increased heterozygosity of hybrid F_1 progeny, acting to mask deleterious recessives, to increase genetic versatility, or both (Shields 1982). There is an emphasis on heterosis in determining fitness, and individuals that are heterozygous at a locus may be more

likely to reproduce successfully than homozygous individuals (Brncic 1954; Shields 1982). Hybridization can also result in a breakdown of intrinsic genomic coadaptation. Combining different genomes through hybridization may disrupt development because potentially new allelic combinations in hybrids have not been subjected to natural selection (Wallace 1981; Templeton 1986). The decreases in fitness that can occur when two genetically divergent or reproductively isolated populations interbreed are commonly called outbreeding depression (Shields 1982), which can result in deleterious shifts in the means of fitness related characters (Lynch 1991). Hybridization, however, is not always associated with increased FA (Ferguson 1986; Gharrett et al. 1999); and studies that have examined levels of FA in hybrids or introgressed populations have not consistently observed reduced developmental stability (reviewed in Leary et al. 1985b). The likelihood that hybridization will disrupt genomic coadaptation depends on the particular populations involved (Leary and Allendorf 1989). Leary et al. (1985b) observed that interspecific hybrids between rainbow trout (*Salmo gairdneri*) and Yellowstone (*Salmo clarki bouvieri*), westslope (*S. c. lewisi*) and coastal (*S. c. clarki*) cutthroat trout have reduced developmental stability relative to their parental species, and Wilkins et al. (1995) reported that developmental stability was reduced in interspecific salmonid hybrids as compared to pure parental species.

Another possible genetic stressor is inbreeding. Because inbreeding lowers both heterozygosity and genomic coadaptation, inbred populations frequently have low developmental stability (Graham 1992) due to a loss of alleles or the expression of 'hidden' deleterious recessives (Emlen 1991). Reduced developmental rate is often

associated with increased differences in bilateral counts of meristic characters, such as fin rays in salmonids; and in the case of inbreeding, it might be that those fish do not have a robust metabolism and fail to develop normally or to produce the genetically determined number of characters in many meristic series (Leary and Allendorf 1989).

The literature does not clearly or completely agree on the effects of hybridization on developmental stability; however, one recurrent theme is that the interference of development patterns is likely to accompany disruption of coadapted gene complexes. FA is the most widely used measure of developmental stability (Van Valen 1962), and one method of demonstrating coadaptation is to crossbreed individuals from geographically isolated populations (Graham and Felley 1985).

Hybrids between spatially separated populations of pink salmon exhibited reduced return rates of adults in the F_1 generation in the odd-year ($p < 0.0001$), but not the even-year, broodline (Gilk et al. 2004). Hybridization reduced survival in both the odd- and even-broodyear second generation returns ($p < 0.005$ and $p < 0.0001$, respectively), however.

The object of this study was to examine the potentially depressive effects of outbreeding on morphological meristic characteristics. Using parents and returns from the previously described experiment, we looked for meristic differences, increased variance in traits and increased levels of FA within F_1 and F_2 generation hybrids relative to control fish and between the F_1 and F_2 generations. Specifically, we analyzed numbers of and variation in left and right branchiostegals, gill rakers of the first and second gill arch, and pectoral fin rays and tested these for evidence of FA. We also looked for evidence of the

effects of sires and dams and their mating combinations used in the crosses on the heritable genetic components of meristics.

STUDY AREA & METHODS

Experiments to produce hybrids between spatially separated populations of pink salmon (*Onchorhynchus gorbuscha*) were initiated in 1996 using a local population of salmon that returns to Auke Creek near Juneau, AK (about 58°23'N, 134°37'W). The donor population for these experiments was Pillar Creek near Kodiak, AK (about 57°47'N, 152°28'W).

Auke Creek is a lake-fed stream with a moderate gradient. It is approximately 350 m in length and has run sizes of pink salmon typically varying between 2,000 and 20,000 per year (Taylor and Lum 2002). Pillar Creek is a reservoir-fed, low-gradient stream that is approximately 1,800 m in length. Its returns of adult pink salmon typically vary between 1,000 and 40,000 per year (Alaska Department of Fish and Game 2000). These two streams are located 1,048 km apart (great circle distance), which suggests that there is very little direct gene flow between the native populations. Since both streams have relatively large and stable pink salmon populations, selection probably favors local adaptation (Adkison 1995).

In the falls of 1996 and 1997, parental crosses were made at Auke Creek. The matings followed a blocked incomplete-factorial statistical design. Pillar Creek females were not included because of concerns over the possible introduction of pathogens to

Auke Creek and marking limitations. Eggs of each Auke Creek female were divided between hybrid and control mating experiments. Semen from Auke Creek males was used to make control crosses, and semen from Pillar Creek males was used for hybrid crosses. Two Auke Creek males and two Pillar Creek males were crossed with each of two Auke Creek females. These sets of crosses were replicated 20 times.

Each family was subdivided into two portions, which were randomly assigned to incubation cells in vertical FAL™ incubators (MariSource, Milton, WA) that were partitioned with acrylic dividers. Hybrid and control families were incubated in different cabinets, and each cabinet had similar high flow rates from a common water source to ensure the maintenance of similar environmental conditions. Temperature records of each incubator document nearly identical temperature regimes. When about 5% of their yolk remained, fry were differentially double fin-clipped (adipose and left or right pelvic) depending on the treatment group. The pelvic side clipped was alternated between hybrid and control groups in different years of the experiment. Approximately 20,000 fish each of hybrid and control crosses were released each spring. Attempts were made to release equal numbers of each family in order to equalize family contributions; however, equal numbers were not always possible. Fry were released at or near the peak of emigration of wild fish in Auke Creek.

Returning adult F_1 individuals were recovered in Auke Creek at a permanent weir maintained by the National Marine Fisheries Service (NMFS) Auke Bay Laboratory just above tidewater; and F_1 returns were bred in 1998 and 1999 to produce second generation fish. Twenty F_2 blocks of controls and twenty F_2 blocks of hybrids, with a two male by

two female design, were made using F_1 returns. Approximately 20,000 fin-marked offspring of each of the F_2 hybrid and control crosses were released at the peak of emigration of wild fish in Auke Creek in mid-April of 1999 and 2000.

Recovered fish were held in pens until they could be processed for information and/or used for breeding experiments; no experimental fish were allowed to spawn in Auke Creek. Each returning fish was numerically tagged, and its experimental group (pelvic fin clip) and gender were noted. For meristic analyses, lengths (mideye to fork of tail) of returning fish were recorded, and fish were frozen at -20°C until left and right branchiostegals, gill rakers of both the first and second gill arches, and pectoral fin rays could be counted (Hubbs and Lagler 1970). Independent counts were made by two individuals, and the few discrepancies were resolved by recounting.

The equality of means of counts and sizes were tested using a two-tailed Student's *t*-test with unequal variances (Sokal and Rohlf 1995). The equality of variances was tested with Levene's test (Millikan and Johnson 1984). Tests of variances were one-tailed tests of the hypothesis that variances of hybrids exceeded variances of controls. Analysis of variance (SYSTAT 2002) was used to test if gender (male or female), cross (hybrid or control), or their interactions affected size or meristic counts. The model used for 1998 and 1999 F_1 or 2000 and 2001 F_2 returns was:

$$Y_{ijk} = \mu + G_i + C_j + G_i * C_j + \varepsilon_{ijk} \quad (1)$$

where Y_{ijk} was the value of the meristic count or size, μ was the mean, G_i was the effect of the *i*th gender, C_j was the effect of the *j*th cross, $G_i * C_j$ was the effect of the interaction of the *i*th gender and the *j*th cross, and ε_{ijk} was the error. The models that

analyzed all returns include year of return (1998, 2000 or 1999, 2001), individual gender (male or female), type of cross (hybrid or control), and their interactions in even and odd broodyears:

$$Y_{ijkl} = \mu + T_i + G_j + C_k + T_i * G_j + T_i * C_k + G_j * C_k + T_i * G_j * C_k + \varepsilon_{ijkl}, \quad (2)$$

where Y_{ijkl} was the value of the meristic count or size, μ was the mean, T_i was the effect of the *i*th year of return, G_j was the effect of the *j*th gender, C_k was the effect of the *k*th cross, $Y_i * G_j$ was the effect of the interaction of the *i*th year of return and the *j*th gender, $Y_i * C_k$ was the effect of the interaction of the *i*th year of return and the *k*th cross, $G_j * C_k$ was the effect of the *j*th gender and the *k*th cross, $Y_i * G_j * C_k$ was the effect of the interaction of the *i*th year of return with the *j*th gender and the *k*th cross, and ε_{ijkl} was the error. Year of return (T), gender (G), and genetic source (C) were fixed effects.

An analysis of asymmetry was conducted to determine the difference in the level of asymmetry in individual meristic characters and see if hybrids were more asymmetric than controls. Asymmetry was quantified by the absolute value of the right minus left sides ($|$ R-L $|$) of each meristic trait. Tests of differences between groups of means (Student's *t*-test) and variances (Levene's test) were one-tailed tests of the hypothesis that asymmetry and its variance in hybrids exceeded that in controls. Composite traits were analyzed to determine the overall level of asymmetry. Composite traits were calculated by summing up all of the absolute right minus left traits (Σ $|$ R-L $|$). Tests of differences between groups of means (Student's *t*-test) and variances (Levene's test) were one-tailed tests of the hypothesis that asymmetry and its variance in hybrids exceeded that in controls.

We also tested for quantitative genetic effects of sire, dam, or their interaction. Previous work (e.g., Gharrett et al. 1999) showed that both means and variances often differ between sexes. Consequently, analyses of male and female progeny were conducted separately in analyses of sire and dam effects. The MIXED model (SAS Version 8.02) was used to test if mating block, cross (hybrid or control), sire, dam, or their interactions had effects on size or meristic counts in 1998 and 1999 F_1 returns:

$$Y_{ijklm} = \mu + B_i + C_j + B_i * C_j + D_{ik} + C_j * D_{ik} + S_{ijl} + D_{ik} * S_{ijl} + \varepsilon_{ijklm} \quad (3)$$

where Y_{ijklm} was the size or count of the meristic trait, μ was the mean, B_i was the effect of the *i*th 2x2 block, C_j was the effect of the *j*th cross, $B_i * C_j$ was the effect of the interaction of the *i*th block and the *j*th cross, D_{ik} was the effect of the *k*th dam within the *i*th block, $C_j * D_{ik}$ was the effect of the interaction of the *j*th cross with the *k*th dam within the *i*th block, S_{ijl} was the effect of the *l*th sire within the *i*th block within the *j*th cross, $D_{ik} * S_{ijl}$ was the effect of the interaction between the *k*th dam and the *l*th sire within the *i*th block within the *j*th cross, and ε_{ijklm} was the error.

In the 2000 and 2001 F_2 returns the model was simplified:

$$Y_{ijklm} = \mu + C_i + B_{ij} + D_{ijk} + S_{ijl} + D_{ijk} * S_{ijl} + \varepsilon_{ijklm} \quad (4)$$

where Y_{ijklm} was the size or count of the meristic trait, μ was the mean, C_i was the effect of the *i*th cross, B_{ij} was the effect of the 2x2 *j*th block within the *i*th cross, D_{ijk} was the effect of the *k*th dam within the *j*th block within *i*th cross, S_{ijl} was the effect of the *l*th sire within the *j*th block within the *i*th cross, $D_{ijk} * S_{ijl}$ was the effect of the interaction between the *k*th dam and the *l*th sire within the *j*th block within the *i*th cross, and ε_{ijklm} was the error. Cross (C) was a fixed effect, and block (B), sire (S), and dam (D) were

random effects. The models differ because in the F_1 experiment, Auke Creek dams were used in both the hybrid and control crosses; but in the F_2 experiment, blocks were made from distinct F_1 control and F_1 hybrid sires and dams.

Because there is no intrinsic biological interpretation of Block or Block*Cross, and these terms were not significant in more tests than would be expected at random, they were removed from analyses giving us size and meristic count models of:

$$Y_{ijkl} = \mu + C_i + D_{ij} + C_i * D_{ij} + S_{ik} + D_{ij} * S_{ik} + \varepsilon_{ijkl} \quad (5)$$

for the 1998 and 1999 F_1 returns, and:

$$Y_{ijkl} = \mu + C_i + D_{ij} + S_{ik} + D_{ij} * S_{ik} + \varepsilon_{ijkl} \quad (6)$$

for the 2000 and 2001 F_2 returns. The "nobound" option was usually included in the MIXED model. When analyses failed to converge or produced 'too many' or 'infinite likelihoods', "nobound" was not invoked, which resulted in constraining all estimates of effects to a non-negative value rather than possibly negative estimates produced by "nobound". This constraint allowed the analyses to converge.

RESULTS

Analysis of meristic counts

Some of the averages and variances of meristic counts of F_1 hybrids, although small, differed significantly from counts of F_1 controls, particularly the average counts of branchiostegals and gill rakers in both males and females in 1998 (Table 1). Fewer differences were observed between F_2 fish (Table 2), although some significant

differences were observed in average counts of pectoral rays in 2001 females, as well as a slight increase in the variance of gill raker counts in both 2000 and 2001 males and females.

Two-way analyses of variance, used to test for the influence of gender and genetic source on size and meristic counts of F_1 fish returning in 1998 and 1999, indicated significant effects of gender and cross for several meristic counts, but relatively little effect by their interaction (Table 3). The effect of gender on right pectoral ray counts was strong in both F_1 years. Results of F_2 comparisons were not as conclusive (Table 4). With the exception of some effects of gender in 2000, and some genetic source effects in 2001, there were very few significant effects for gender, genetic source, or the interaction term in the F_2 returns.

The data from the two generations of each broodline were analyzed to test for interannual effects. Three-way ANOVAs for the influence of calendar year, gender, genetic source, and their interactions indicated that year was important to size and meristic counts in both the even and odd broodlines; but that gender only affected size and meristic counts in the even broodline (Table 5). Genetic source had little significance in all years, whereas interactions had some significance, mainly occurring when year was one of the terms.

Analysis of asymmetry

Little difference was observed between hybrids and controls in averages or variances of levels of asymmetry in both the F_1 and F_2 generations (Tables 6 and 7

respectively). Branchiostegal number clearly had directional asymmetry ($p < 0.0003$ overall, Student's *t*-test with unequal variances), averaging nearly one more branchiostegal on the left side than on the right (Tables 1 and 2), but the magnitude and variance were only slightly significant between hybrids and controls in each year. Pectoral rays also showed directional asymmetry, but this was mainly observed in 1998 F_1 returns ($p < 0.003$, Student's *t*-test with unequal variances), and again the magnitude and variance do not differ significantly between hybrids and controls. In the composite trait analysis (Table 8, combined FA index), no significant differences were observed between hybrids and controls in either the even or odd F_1 and F_2 generations.

Genetic components in meristic characters

Tests of the influence of the effects of cross, sire, dam, or their interactions on the sizes and meristic counts of F_1 fish returning in 1998 and 1999, indicated significant influence of cross, sire, and dam for several meristic counts, but no effect from the interaction terms in 1998 (Table 9). Dam and cross influenced counts of gill rakers. In 1999, cross and dam influenced several traits and some effects of sire and the interaction terms were also observed. Results of F_2 comparisons were similar to those in the F_1 (Table 10). Dam, sire, and their interaction, rather than cross, seemed to influence gill raker counts in 2000, whereas cross, dam, and sire effects influenced counts of pectoral rays and branchiostegals in 2001.

DISCUSSION

We observed no obvious influence of outbreeding depression on F_1 or F_2 hybrids between members of spatially separated pink salmon populations from Southeast Alaska and Kodiak Island. This study included a much larger sample than that examined in a previous study of inter-broodline pink salmon hybrids (Gharrett et al. 1999), but even with its much larger data set, our results are generally consistent with previous studies. Some variation in size and meristic counts of returning F_1 and F_2 hybrids of spatially separated populations of pink salmon, relative to controls, was observed; however, no evidence was seen for increased fluctuating asymmetry in hybrids versus controls. Directional asymmetry was highly significant for branchiostegals, and, to a lesser degree, for pectoral fin ray counts, which makes sense because positioning of the heart during cardiac development is directionally asymmetric, and these structures enclose it. This is also consistent with other studies; both Berg et al. (1997) and Gharrett et al. (1999) observed that branchiostegal number has a directional asymmetry, averaging nearly one more branchiostegal on the left side than the right.

No single character consistently showed a dam, sire, or cross effect in general except gill rakers. The dam effect, however, appears to have more influence than the sire. One explanation for the dam effects could be egg quality of individual females at the onset of the experiment. All eggs were maintained in a common environment, so it is likely that any environmental variations would affect all offspring the same. However, the health and fitness of some families may have been lower at the onset because of egg

quality differences (maternal effect). These are somewhat different results from those obtained by Smoker et al. (1994). They found that variation in size had a significant genetic basis in pink salmon, particularly in males, and that the sire effect was specifically significant. One reason our results may differ from theirs could be due to the different environments experienced by fish in the two experiments.

Gharrett and Smoker (1991) looked at hybridizations of even- and odd-broodyear pink salmon. They observed an increase in bilateral asymmetry in F_2 hybrids, although no controls were available for comparison. When these experiments were repeated to include controls (Gharrett et al. 1999), however, they failed to detect an increase in FA of paired meristic counts in either F_1 or F_2 hybrids. Although no significant differences in bilateral asymmetry were observed, size and some meristic counts of hybrids exceeded measurements of controls, suggesting heterosis or hybrid vigor for those traits. In another study, Ferguson (1986) observed that the developmental stability (inferred from FA in four meristic characters) of first generation hybrids between hatchery strains of rainbow trout (*Salmo gairdneri*) was higher relative to three pure parental strains in two of the three reciprocal hybrid pairs. The third hybrid pair showed reduced, but not significantly lower developmental stability.

There is as yet no consensus on the effect hybridization has on developmental stability. Leary et al. (1985a) reported a significant correlation between the average heterozygosity of each family at allozyme loci and the average number of asymmetric traits per individual, as well as a strong correlation between heterozygosity at those loci and decreased FA in several salmonid populations. Graham and Felley (1985) and Leary

et al. (1985b) observed increased FA in both introgressed populations and interspecific hybrids, suggesting that the decreased developmental stability of these hybrids may be the result of disruption of genomic coadaptation (Ferguson 1986). Hochwender and Fritz (1999) also observed that *Salix* hybrids had significantly greater FA than did parental species, and that F_2 hybrids had marginally greater FA than did F_1 hybrids. Both of these results suggest that genetic stress through disruption of coadapted gene complexes had a much greater influence on developmental stability than did heterozygosity. Because half of their genome came from each parental species, hybrid individuals had greater heterozygosity, and should have had lower FA than parental species if the predictions of the heterozygosity hypothesis were met. Instead, the observed pattern of greater FA in the hybrid taxa suggests that the genetic balance within each species was disrupted through hybridization (Hochwender and Fritz 1999).

Developmental stability, through canalization, refers to an individual's ability to withstand or buffer developmental accidents of genetic or environmental origin (Graham and Felley 1985). In general, evidence suggests that disruption of coadapted gene complexes increases fluctuating asymmetry, reducing developmental stability, with the degree of divergence between parental species and the recentness of hybridization modifying the effect of genetic disruption (Hochwender and Fritz 1999). In this study, even though hybrid parent populations were separated by a large distance and differed genetically (Gilk et al. 2004), no evidence of increased fluctuating asymmetry was observed. It is possible that heterosis in the F_1 generations was able to compensate for any disruption of coadapted genomes (Shields 1982; Geiger 1988; Emlen 1991; Lynch

1991). No differences in FA were observed in the F_2 generations. Graham (1992) suggested that developmental stability depends on a balance between heterozygosity and coadaptation. Another thought is that the low genetic (sire) effect indicates little additive variation remains, and little sire by dam interaction indicates there is little nonadditive variation, so it is possible that the homeotic process is conserved and has diverged little in the time since these populations were established after the glacial recession. Consequently, there insufficient differences in the coadapted gene complexes to increase fluctuating asymmetry through hybridization, even though a decrease in survival between hybrids and controls was seen (Gilk et al. 2004).

REFERENCES

Adkison, M. D. 1995. Population differentiation in Pacific salmon: local adaptation, genetic drift, or the environment? Canadian Journal of Fisheries and Aquatic Sciences 52(12):2762-2777.

Alaska Department of Fish and Game, Kodiak. 2000. Stream survey data of Pillar Creek, Kodiak, AK from 1968 to 2000. Unpublished data.

Barlow, G. W. 1961. Causes and significance of morphological variation in fishes. Systematic Zoology 10(3):105-117.

Berg, O. K., M. D. Adkison, and T. P. Quinn. 1997. Bilateral asymmetry, sexual dimorphism, and nematode parasites in mature male sockeye salmon *Oncorhynchus nerka*. Northwest Science 71(4):305-312.

Brncic, D. 1954. Heterosis and the integration of the genotype in geographic populations of *Drosophila pseudoobscura*. Genetics 39:77-88.

Bryden, C. A. and D. D. Heath. 2000. Heritability of fluctuating asymmetry for multiple traits in chinook salmon (*Oncorhynchus tshawytscha*). Canadian Journal of Fisheries and Aquatic Sciences 57(11):2186-2192.

Clarke, G. M. 1993. The genetic basis of developmental stability. I. Relationships between stability, heterozygosity and genomic coadaptation. Genetica 89:15-23.

Emlen, J. M. 1991. Heterosis and outbreeding depression: a multi-locus model and an application to salmon production. Fisheries Research 12:187-212.

Ferguson, M. M. 1986. Developmental stability of rainbow trout hybrids: genomic coadaptation or heterozygosity? Evolution 40(2):323-330.

Geiger, H. H. 1988. Epistasis and heterosis. Pages 395-399 *in* B. S. Weir, E. E. Eisen, M. M. Goodman, and G. Namkoong, editors. Proceedings of the 2nd International Conference on Quantitative Genetics. Sinauer Associates, Inc., Sunderland, MA.

Gharrett, A. J. and W. W. Smoker. 1991. Two generations of hybrids between even- and odd-year pink salmon (*Oncorhynchus gorbuscha*): a test for outbreeding depression? Canadian Journal of Fisheries and Aquatic Sciences 48(9):1744-1749.

Gharrett, A. J., W. W. Smoker, R. R. Reisenbichler, and S. G. Taylor. 1999. Outbreeding depression in hybrids between odd- and even-broodyear pink salmon. Aquaculture 173:117-129.

Gilk, S. E., I. A. Wang, C. L. Hoover, W. W. Smoker, S. G. Taylor, A. K. Gray, and A. J. Gharrett. 2004. Outbreeding depression in hybrids between spatially separated pink salmon, *Oncorhynchus gorbuscha,* populations: marine survival, homing ability, and variability in family size. Environmental Biology of Fishes 69:287-297.

Graham, J. H. 1992. Genomic coadaptation and developmental stability in hybrid zones. Acta Zoologica Fennica 191:121-131.

Graham, J. H. and J. D. Felley. 1985. Genomic coadaptation and developmental stability within introgressed populations of *Enneacanthus gloriosus* and *E. obesus* (Pisces, Centrarchidae). Evolution 39(1):104-114.

Hochwender, C. G. and R. S. Fritz. 1999. Fluctuating asymmetry in a *Salix* hybrid system: the importance of genetic versus environmental causes. Evolution 53(2):408-416.

Hubbs, C. L. and K. F. Lagler. 1970. Fishes of the Great Lakes Region. University of Michigan Press, Bloomfield Hills, MI.

Leary, R. F. and F. W. Allendorf. 1989. Fluctuating asymmetry as an indicator of stress: implications for conservation biology. Trends in Ecology & Evolution 4(7):214-217.

Leary, R. F., F. W. Allendorf, and K. L. Knudsen. 1985a. Inheritance of meristic variation and the evolution of developmental stability in rainbow trout. Evolution 39(2):308-314.

Leary, R. F., F. W. Allendorf, and K. L. Knudsen. 1985b. Developmental instability and high meristic counts in interspecific hybrids of salmonid fishes. Evolution 39(6):1318-1326.

Lynch, M. 1991. The genetic interpretation of inbreeding depression and outbreeding depression. Evolution 45(3):622-629.

Millikan, G. A. and D. E. Johnson. 1984. Analysis of Messy Data, Volume I: Designed Experiments. Lifetime Learning Publishers, Belmont, CA.

Møller, A. P. 1997. Developmental stability and fitness: a review. The American Naturalist 149(5):916-932.

Moyle, P. B. and J. J. Cech Jr. 2000. Fishes: an introduction to ichthyology. Prentice-Hall, Inc., Upper Saddle River, NJ.

Palmer, A. R. and C. Strobeck. 1986. Fluctuating asymmetry: measurement, analysis, patterns. Annual Review of Ecology and Systematics 17:391-421.

Palmer, A. R. and C. Strobeck. 1992. Fluctuating asymmetry as a measure of developmental stability: Implications of non-normal distributions and power of statistical tests. Acta Zoologica Fennica 191:57-72.

SAS Version 8.02. 1999. SAS Institute Inc., Cary, NC.

Shields, W. M. 1982. Philopatry, inbreeding, and the evolution of sex. State University of New York Press, Albany, NY.

Smoker, W. W., A. J. Gharrett, M. S. Stekoll, and J. E. Joyce. 1994. Genetic analysis of size in an anadromous population of pink salmon. Canadian Journal of Fisheries and Aquatic Sciences 51(Suppl. 1):9-15.

Sokal, R. R. and F. J. Rohlf. 1995. Biometry: the principles and practice of statistics in biological research, 3rd edition. W. H. Freeman and Co., New York.

Soulé, M. E. 1979. Heterozygosity and developmental stability: another look. Evolution 33(1):396-401.

SYSTAT. 2002. SYSTAT 10.2 for Windows. Point Richmond, CA.

Tåning, Å. V. 1952. Experimental study of meristic characters in fishes. Biological Reviews 27:169-193.

Taylor, S. G. and J. L. Lum. 2002. Auke Creek Weir 2001 Annual Report, Operations, Fish Counts, and Historical Summaries. Unpublished Report. National Marine Fisheries Service, Auke Bay Laboratory, 11305 Glacier Highway, Juneau, AK 99801-8626.

Templeton, A. R. 1986. Coadaptation and outbreeding depression. Pages 105-116 *in* M. E. Soulé, editor. Conservation biology: the science of scarcity and diversity. Sinauer Associates, Inc., Sunderland, MA.

Van Valen, L. 1962. A study of fluctuating asymmetry. Evolution 16(2):125-142.

Waddington, C. H. 1942. Canalization of development and the inheritance of acquired characters. Nature 150(3811):563-565.

Wallace, B. 1981. Basic population genetics. Columbia University Press, New York, NY.

Wilkins, N. P., E. Gosling, A. Curatolo, A. Linnane, C. Jordan, and H. P. Courtney. 1995. Fluctuating asymmetry in Atlantic salmon, European trout, and their hybrids, including triploids. Aquaculture 137(1-4):77-85.

Table 1. Meristic characters [mean ($\bar{x}$), variance (s^2), and sample size (n)] of 1998 and 1999 (F_1) controls and hybrids between spatially separated populations of pink salmon. Comparisons between hybrids and controls were two-tailed t-tests for means and one-tailed t-tests for variances.

							Rakers on 1st Gill Arch				Rakers on 2nd Gill Arch			
		Length	Pectoral Rays		Branchiostegals		Upper		Lower		Upper		Lower	
Source		MEFL	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right
1998 (F_1) Females														
Control:	$\bar{x}$	459.1	15.99	16.22	**12.67**	**12.07**	**11.69**	**11.68**	18.34	18.49	10.49	10.43	**17.50**	17.54
Hybrid:	$\bar{x}$	460.1	15.96	16.12	**12.92**[c]	**12.25**[b]	**11.86**[a]	**11.85**[a]	18.48	18.46	10.58	10.47	**17.70**[b]	17.61
Control:	s^2	450.7	0.488	0.554	0.454	0.416	0.497	0.434	0.870	0.979	0.450	0.380	0.550	0.680
Hybrid:	s^2	515.4	0.566	0.579	0.518	0.360	0.429	0.621	1.014	0.912	0.449	0.455	0.603	0.631
Control:	n	122	122	122	122	122	122	122	122	122	122	122	122	122
Hybrid:	n	119	119	119	119	119	119	119	119	119	119	119	119	119
1998 (F_1) Males														
Control:	$\bar{x}$	**447.7**	16.17	16.43	**12.78**	**12.17**	11.73	**11.70**	**18.45**	18.63	10.61	10.58	17.63	**17.61**
Hybrid:	$\bar{x}$	**462.9**[d]	16.10	16.37	**12.94**[a]	**12.36**[b]	11.84	**11.96**[b]	**18.68**[b]	18.63	10.59	10.61	17.69	**17.85**[b]
Control:	s^2	1050.7	0.437	0.511	0.382	**0.338**	0.427	0.543	0.531	0.690	0.328	0.298	0.725	0.714
Hybrid:	s^2	1094.5	0.437	0.506	0.443	**0.406**[b]	0.468	0.542	0.724	0.703	0.439	0.396	0.506	0.481
Control:	n	115	115	115	115	115	115	115	115	115	115	115	115	115
Hybrid:	n	104	104	104	104	104	104	104	104	104	104	104	104	104

Table 1 continued.

Source		Length	Pectoral Rays		Branchiostegals		Rakers on 1st Gill Arch				Rakers on 2nd Gill Arch			
							Upper		Lower		Upper		Lower	
		MEFL	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right
1999 (F_1) Females														
Control:	$\bar{x}$	438.4	15.84	15.96	12.89	12.27	12.33	12.25	18.89	18.88	10.73	10.64	**17.75**	17.85
Hybrid:	$\bar{x}$	439.3	15.79	15.86	12.91	12.21	12.18	12.09	18.95	19.02	10.67	10.70	**17.89**[a]	17.83
Control:	s^2	475.7	0.489	**0.322**	0.378	0.331	0.502	0.467	0.810	0.668	0.346	0.334	0.409	0.523
Hybrid:	s^2	499.6	0.273	**0.350**[a]	0.330	0.369	0.407	0.484	0.666	0.653	0.345	0.365	0.450	0.510
Control:	n	137	137	137	137	137	137	137	136	136	137	137	137	137
Hybrid:	n	131	131	131	131	131	131	131	131	131	131	131	131	131
1999 (F_1) Males														
Control:	$\bar{x}$	444.2	15.98	16.09	13.11	12.39	12.43	12.38	19.09	19.13	10.88	10.85	17.87	17.99
Hybrid:	$\bar{x}$	437.0	15.92	16.14	12.91	12.33	12.16	12.17	18.84	18.90	10.70	10.70	17.72	17.73
Control:	s^2	**905.2**	0.429	0.321	0.358	0.346	0.623	0.508	0.885	0.783	**0.316**	0.318	0.525	0.606
Hybrid:	s^2	**1207.9**[b]	0.262	0.361	0.417	0.353	0.411	0.491	0.559	0.758	**0.324**[c]	0.250	0.687	0.493
Control:	n	171	171	171	171	171	171	171	171	171	171	171	171	171
Hybrid:	n	109	109	109	109	109	109	109	109	109	109	109	109	109

[a]$P \leq 0.10$, [b]$P \leq 0.05$, [c]$P \leq 0.01$, or [d]$P \leq 0.001$.
Tests were not corrected for multiple testing.

Table 2. Meristic characters [mean ($\bar{x}$), variance (s^2), and sample size (n)] of 2000 and 2001 (F_2) controls and hybrids between spatially separated populations of pink salmon. Comparisons between hybrids and controls were two-tailed t-tests for means and one-tailed t-tests for variances.

							Rakers on 1st Gill Arch				Rakers on 2nd Gill Arch			
		Length	Pectoral Rays		Branchiostegals		Upper		Lower		Upper		Lower	
Source		MEFL	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right
2000 (F_2) Females														
Control:	$\bar{x}$	457.0	15.85	15.86	13.09	12.37	11.99	11.98	18.64	18.71	10.68	10.7	17.64	17.7
Hybrid:	$\bar{x}$	456.3	15.86	15.98	13.02	12.29	12.07	12.02	18.73	18.63	10.57	10.71	17.77	17.82
Control:	s^2	320.6	0.408	0.283	0.387	0.398	0.523	0.395	**0.558**	**0.509**	0.523	0.282	1.604	0.468
Hybrid:	s^2	365.2	0.306	0.309	0.418	0.571	0.286	0.491	**1.872**[b]	**1.766**[b]	0.249	0.281	0.800	0.840
Control:	n	87	87	87	87	87	87	87	87	87	87	87	87	87
Hybrid:	n	56	56	56	56	56	56	56	56	56	56	56	56	56
2000 (F_2) Males														
Control:	$\bar{x}$	**442.7**	16.01	16.06	13.01	12.26	12.02	11.95	18.83	18.74	10.66	10.67	17.76	17.79
Hybrid:	$\bar{x}$	**452.8**[a]	16.06	16.06	13.02	12.42	11.94	11.88	18.81	18.94	10.56	10.56	17.81	17.63
Control:	s^2	964.8	0.318	0.361	0.318	0.428	**0.541**	0.468	**0.710**	**0.498**	0.603	0.387	0.563	**0.473**
Hybrid:	s^2	564.8	0.315	0.358	0.404	0.333	**0.698**[b]	0.537	**0.922**[a]	**1.251**[d]	0.294	0.294	0.581	**0.750**[c]
Control:	n	86	86	86	86	86	86	86	86	86	86	86	86	86
Hybrid:	n	48	48	48	48	48	48	48	48	48	48	48	48	48

Table 2 continued.

Source		Length	Pectoral Rays		Branchiostegals		Rakers on 1st Gill Arch				Rakers on 2nd Gill Arch			
							Upper		Lower		Upper		Lower	
		MEFL	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right
2001 (F_2) Females														
Control:	$\bar{x}$	465.76	**15.69**	**15.72**	13.16	12.45	12.25	12.27	19.06	19.24	10.70	10.70	18.08	**17.96**
Hybrid:	$\bar{x}$	470.71	**16.02**[d]	**15.92**[b]	13.11	12.53	12.24	12.36	19.14	19.26	10.85	10.80	18.17	**18.24**[b]
Control:	s^2	399.22	0.312	0.300	0.352	0.328	0.539	0.412	**0.696**	0.612	0.347	0.367	**0.441**	0.562
Hybrid:	s^2	534.58	0.323	0.286	0.250	0.345	0.617	0.481	**0.746**[a]	0.748	0.500	0.284	**0.633**[c]	0.525
Control:	*n*	104	104	104	104	104	104	104	104	104	104	104	104	104
Hybrid:	*n*	66	66	66	66	66	66	66	65	66	66	66	66	66
2001 (F_2) Males														
Control:	$\bar{x}$	479.82	15.76	15.79	13.19	12.43	12.31	12.32	19.04	19.09	**10.75**	**10.73**	**17.86**	18.09
Hybrid:	$\bar{x}$	465.21	15.79	15.84	12.92	12.33	12.32	12.42	19.21	19.27	**10.95**[b]	**10.92**[a]	**18.15**[b]	18.11
Control:	s^2	1333.2	0.409	0.375	0.401	0.359	0.479	**0.352**	**0.653**	0.720	0.301	0.404	**0.438**	0.646
Hybrid:	s^2	1088.5	0.277	0.306	0.382	0.363	0.469	**0.581**[c]	**0.666**[b]	0.757	0.358	0.438	**0.824**[d]	0.571
Control:	*n*	108	108	108	108	108	108	108	108	108	108	108	108	108
Hybrid:	*n*	73	73	73	73	73	73	73	73	73	73	73	73	73

[a]$P \leq 0.10$, [b]$P \leq 0.05$, [c]$P \leq 0.01$, or [d]$P \leq 0.001$.
Tests were not corrected for multiple testing.

Table 3. Significance of tests (ANOVA) for effects on size and meristics of gender (G), genetic source (C), and their interaction (G x C) for (upper) 1998 F_1 and (lower) 1999 F_1 hybrid and control returns.

Character	G	C	G x C
Length (MEF)		**	**
Pectoral Rays (L)	*		
Pectoral rays (R)	***		
Branchiostegals (L)		***	
Branchiostegals (R)		**	
Gill Rakers			
1st arch, Upper (L)		*	
1st arch, Upper (R)		**	
1st arch, Lower (L)		*	
1st arch, Lower (R)			
2nd arch, Upper (L)			
2nd arch, Upper (R)	*		
2nd arch, Lower (L)			
2nd arch, Lower (R)	*	*	

Character	G	C	G x C
Length (MEF)			
Pectoral Rays (L)	*		
Pectoral rays (R)	***		
Branchiostegals (L)	*		*
Branchiostegals (R)	*		
Gill Rakers			
1st arch, Upper (L)		***	
1st arch, Upper (R)		**	
1st arch, Lower (L)			*
1st arch, Lower (R)			*
2nd arch, Upper (L)		*	
2nd arch, Upper (R)	*		*
2nd arch, Lower (L)			*
2nd arch, Lower (R)		*	

L is a count on the left side, R is on the right side, and MEF is mideye to fork of tail.
* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.
Tests do not include corrections for multiple tests.

Table 4. Significance of tests (ANOVA) for effects on size and meristics of gender (G), genetic source (C), and their interaction (G x C) for (upper) 2000 F_2 and (lower) 2001 F_2 hybrid and control returns.

Character	G	C	G x C
Length (MEF)	**		
Pectoral Rays (L)	*		
Pectoral rays (R)			
Branchiostegals (L)			
Branchiostegals (R)			
Gill Rakers			
1st arch, Upper (L)			
1st arch, Upper (R)			
1st arch, Lower (L)			
1st arch, Lower (R)			
2nd arch, Upper (L)			
2nd arch, Upper (R)			
2nd arch, Lower (L)			
2nd arch, Lower (R)			

Character	G	C	G x C
Length (MEF)			**
Pectoral Rays (L)		**	*
Pectoral rays (R)		*	
Branchiostegals (L)		*	
Branchiostegals (R)			
Gill Rakers			
1st arch, Upper (L)			
1st arch, Upper (R)			
1st arch, Lower (L)			
1st arch, Lower (R)			
2nd arch, Upper (L)		**	
2nd arch, Upper (R)		*	
2nd arch, Lower (L)		*	
2nd arch, Lower (R)			

L is a count on the left side, R is on the right side, and MEF is mideye to fork of tail.
* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.
Tests do not include corrections for multiple tests.

Table 5. Significance of tests (ANOVA) for effects on size and meristics of year of return (Y), gender (G), genetic source (C), and their interactions for (upper) 1998 F_1 and 2000 F_2 and (lower) 1999 F_1 and 2001 F_2 hybrid and control returns.

Character	Y	G	C	Y x G	Y x C	G x C	Y x G x C
Length (MEF)	*	***	**			**	
Pectoral Rays (L)	*	***					
Pectoral rays (R)	***	***					
Branchiostegals (L)	***				*		
Branchiostegals (R)	*		*				
Gill Rakers							
1st arch, Upper (L)	***						
1st arch, Upper (R)	**				*		
1st arch, Lower (L)	***	*					
1st arch, Lower (R)	**	*					
2nd arch, Upper (L)							
2nd arch, Upper (R)	**			*			
2nd arch, Lower (L)							
2nd arch, Lower (R)							

Character	Y	G	C	Y x G	Y x C	G x C	Y x G x C
Length (MEF)	***		*			***	
Pectoral Rays (L)				*	**		
Pectoral rays (R)	***	*		**			
Branchiostegals (L)	***		**	*		**	
Branchiostegals (R)	***			**			
Gill Rakers			*				
1st arch, Upper (L)					*		
1st arch, Upper (R)	**				**		
1st arch, Lower (L)	**						
1st arch, Lower (R)	***						*
2nd arch, Upper (L)		*			***		
2nd arch, Upper (R)		*			*		
2nd arch, Lower (L)	***						*
2nd arch, Lower (R)	***				**	*	

L is a count on the left side, R is on the right side, and MEF is mideye to fork of tail.
* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.
Tests do not include corrections for multiple tests.

Table 6. Proportion of individuals asymmetric for meristic characters [mean ($\bar{x}$), variance (s^2), and sample size (n)] of 1998 and 1999 (F_1) hybrids between spatially separated populations of pink salmon and their controls. Comparisons between hybrids and controls were one-tailed t-tests for means and one-tailed t-tests for variances.

				Rakers on 1st Gill Arch		Rakers on 2nd Gill Arch	
Source		Pectoral Rays	Branchiostegals	Upper	Lower	Upper	Lower
1998 (F_1) Females							
Control:	$\bar{x}$	0.46	0.63	0.45	0.55	0.37	0.63
Hybrid:	$\bar{x}$	0.45	0.68	0.43	0.59	0.39	0.50
Control:	s^2	0.30	0.33	0.30	0.40	0.25	0.43
Hybrid:	s^2	0.35	0.34	0.37	0.43	0.31	0.32
Control:	n	122	122	122	122	122	122
Hybrid:	n	119	119	119	119	119	119
1998 (F_1) Males							
Control:	$\bar{x}$	0.44	0.66	0.48	0.58	0.30	0.69
Hybrid:	$\bar{x}$	0.38	0.64	0.51	0.60	0.27	0.46
Control:	s^2	0.30	0.33	0.30	0.35	0.21	0.41
Hybrid:	s^2	0.32	0.35	0.31	0.40	0.22	0.33
Control:	n	115	115	115	115	115	115
Hybrid:	n	104	104	104	104	104	104

Table 6 continued.

Source		Pectoral Rays	Branchiostegals	Rakers on 1st Gill Arch Upper	Rakers on 1st Gill Arch Lower	Rakers on 2nd Gill Arch Upper	Rakers on 2nd Gill Arch Lower
1999 (F_1) Females							
Control:	$\bar{x}$	0.26	0.65	0.33	0.68	0.39	0.53
Hybrid:	$\bar{x}$	0.22	0.71	0.39	0.60	0.27	0.53
Control:	s^2	0.28	0.33	0.24	0.40	0.24	0.30
Hybrid:	s^2	0.17	0.38	0.35	0.37	0.20	0.33
Control:	n	137	137	137	136	137	137
Hybrid:	n	131	131	131	131	131	131
1999 (F_1) Males							
Control:	$\bar{x}$	0.29	0.72	0.44	0.58	0.30	0.56
Hybrid:	$\bar{x}$	0.31	0.65	0.34	0.55	0.28	0.59
Control:	s^2	0.39	**0.36**	0.35	0.37	0.21	0.38
Hybrid:	s^2	0.27	**0.30**[b]	0.28	0.38	0.22	0.49
Control:	n	171	171	171	171	171	171
Hybrid:	n	109	109	109	109	109	109

[a]$P \leq 0.10$, [b]$P \leq 0.05$, [c]$P \leq 0.01$, or [d]$P \leq 0.001$.
Tests were not corrected for multiple testing.

Table 7. Proportion of individuals asymmetric for meristic characters [mean ($\bar{x}$), variance (s^2), and sample size (n)] of 2000 and 2001 (F_2) hybrids between spatially separated populations of pink salmon and their controls. Comparisons between hybrids and controls were one-tailed t-tests for means and one-tailed t-tests for variances.

Source		Pectoral Rays	Branchiostegals	Rakers on 1st Gill Arch Upper	Rakers on 1st Gill Arch Lower	Rakers on 2nd Gill Arch Upper	Rakers on 2nd Gill Arch Lower
2000 (F_2) Females							
Control:	$\bar{x}$	0.15	0.77	0.43	0.64	0.41	0.70
Hybrid:	$\bar{x}$	0.20	0.77	0.38	0.75	0.39	0.52
Control:	s^2	**0.15**	0.34	0.34	0.33	0.45	1.14
Hybrid:	s^2	**0.16**[b]	0.47	0.24	0.41	0.24	0.47
Control:	n	87	87	87	87	87	87
Hybrid:	n	56	56	56	56	56	56
2000 (F_2) Males							
Control:	$\bar{x}$	**0.12**	0.76	0.37	0.66	0.34	0.62
Hybrid:	$\bar{x}$	**0.21**[a]	0.65	0.40	0.67	0.29	0.48
Control:	s^2	0.10	0.40	0.26	0.30	0.39	0.31
Hybrid:	s^2	0.17	0.36	0.29	0.31	0.21	0.34
Control:	n	86	86	86	86	86	86
Hybrid:	n	48	48	48	48	48	48

Table 7 continued.

Source		Pectoral Rays	Branchiostegals	Rakers on 1st Gill Arch Upper	Rakers on 1st Gill Arch Lower	Rakers on 2nd Gill Arch Upper	Rakers on 2nd Gill Arch Lower
2001 (F_2) Females							
Control:	$\bar{x}$	0.16	0.73	**0.35**	0.64	0.33	0.58
Hybrid:	$\bar{x}$	0.21	0.58	**0.52**[b]	0.48	0.41	0.50
Control:	s^2	**0.14**	0.28	**0.29**	0.39	0.24	0.34
Hybrid:	s^2	**0.17**[a]	0.28	**0.53**[c]	0.35	0.43	0.32
Control:	*n*	104	104	104	104	104	104
Hybrid:	*n*	66	66	66	65	66	66
2001 (F_2) Males							
Control:	$\bar{x}$	0.29	0.79	0.47	0.63	0.37	0.62
Hybrid:	$\bar{x}$	0.15	0.62	0.36	0.59	0.41	0.51
Control:	s^2	0.24	0.34	0.29	0.37	0.24	0.35
Hybrid:	s^2	0.13	0.30	0.26	0.38	0.25	0.36
Control:	*n*	108	108	107	108	108	108
Hybrid:	*n*	73	73	73	73	73	73

[a]$P \leq 0.10$, [b]$P \leq 0.05$, [c]$P \leq 0.01$, or [d]$P \leq 0.001$.
Tests were not corrected for multiple testing.

Table 8. Composite trait analysis of meristic characters [mean ($\bar{x}$), variance (s^2), and sample size (n)] of 1998 and 1999 (F_1) and 2000 and 2001 (F_2) hybrids between spatially separated populations of pink salmon and their controls. Comparisons between hybrids and controls were one-tailed t-tests for means and one-tailed t-tests for variances.

Source		All Traits
1998 (F_1)		
Control:	$\bar{x}$	3.122
	s^2	1.845
	n	237
Hybrid:	$\bar{x}$	2.955
	s^2	1.989
	n	223

Source		All Traits
1999 (F_1)		
Control:	$\bar{x}$	2.870
	s^2	1.996
	n	308
Hybrid:	$\bar{x}$	2.721
	s^2	2.010
	n	240

Source		All Traits
2000 (F_2)		
Control:	$\bar{x}$	2.983
	s^2	2.273
	n	173
Hybrid:	$\bar{x}$	2.856
	s^2	1.697
	n	104

Source		All Traits
2001 (F_2)		
Control:	$\bar{x}$	3.033
	s^2	2.421
	n	212
Hybrid:	$\bar{x}$	2.799
	s^2	4.858
	n	139

[a]$P \leq 0.10$, [b]$P \leq 0.05$, [c]$P \leq 0.01$, or [d]$P \leq 0.001$.

Table 9. Significance of tests (SAS MIXED) for heritable effects on size and meristics of cross, sire, dam, and their interactions for (upper) 1998 and (lower) 1999 F_1 hybrid and control female and male returns.

	Cross		Dam		Sire		Cross*Dam		Sire*Dam	
Character	F	M	F	M	F	M	F	M	F	M
Length (MEF)		*								
Pectoral Rays (L)										
Pectoral rays (R)				*						
Branchiostegals (L)	*				*	*				
Branchiostegals (R)					***					
Gill Rakers										
1st arch, Upper (L)	*		**							
1st arch, Upper (R)			*							
1st arch, Lower (L)		*								
1st arch, Lower (R)										
2nd arch, Upper (L)			*							
2nd arch, Upper (R)		**	*							
2nd arch, Lower (L)			**							
2nd arch, Lower (R)										

	Cross		Dam		Sire		Cross*Dam		Sire*Dam	
Character	F	M	F	M	F	M	F	M	F	M
Length (MEF)			***		*	*	***			
Pectoral Rays (L)					*					
Pectoral rays (R)										
Branchiostegals (L)		*	*		*					
Branchiostegals (R)						*				
Gill Rakers										
1st arch, Upper (L)		**		**						
1st arch, Upper (R)			*							
1st arch, Lower (L)		*								
1st arch, Lower (R)										
2nd arch, Upper (L)			*	*						
2nd arch, Upper (R)		**		**				**		
2nd arch, Lower (L)										
2nd arch, Lower (R)		***		*					*	

L is a count on the left side, R is on the right side, and MEF is mideye to fork of tail.
* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.
Tests do not include corrections for multiple tests.

Table 10. Significance of tests (SAS MIXED) for heritable effects on size and meristics of cross, sire, dam, and their interactions for (upper) 2000 and (lower) 2001 F_2 hybrid and female and male returns.

	Cross		Dam		Sire		Sire*Dam	
Character	F	M	F	M	F	M	F	M
Length (MEF)					*			
Pectoral Rays (L)								
Pectoral rays (R)				*				***
Branchiostegals (L)								
Branchiostegals (R)								
Gill Rakers								
1st arch, Upper (L)								
1st arch, Upper (R)								
1st arch, Lower (L)								
1st arch, Lower (R)				*				
2nd arch, Upper (L)				***	*			
2nd arch, Upper (R)			*		**		**	
2nd arch, Lower (L)				*		*		**
2nd arch, Lower (R)								

	Cross		Dam		Sire		Sire*Dam	
Character	F	M	F	M	F	M	F	M
Length (MEF)			*			*		
Pectoral Rays (L)	*							
Pectoral rays (R)				*	**			
Branchiostegals (L)		*	*		*		**	
Branchiostegals (R)								
Gill Rakers								
1st arch, Upper (L)			*					
1st arch, Upper (R)								
1st arch, Lower (L)								
1st arch, Lower (R)								
2nd arch, Upper (L)								
2nd arch, Upper (R)			**					
2nd arch, Lower (L)			*					
2nd arch, Lower (R)			*					

L is a count on the left side, R is on the right side, and MEF is mideye to fork of tail.
* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.
Tests do not include corrections for multiple tests.